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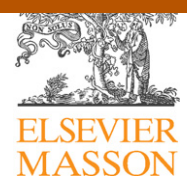

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CLINICAL RESEARCH

Testosterone improves cardiac function and alters angiotensin II receptors in isoproterenol-induced heart failure

La testostérone améliore la fonction cardiaque et altère les récepteurs à l'angiotensine II dans l'insuffisance cardiaque induite par l'isoprotérénol

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KEYWORDS
Testosterone;
Heart failure;
Angiotensin II receptor;
Apoptosis;
Fibrosis

Summary
Background. — The renin-angiotensin-aldosterone system is known to play an important role in the pathophysiology and development of heart failure. Several studies have reported the benefits of testosterone in heart failure. However, the mechanisms of testosterone-induced effects on heart failure require further study.
Aims. — To determine the effects of castration and testosterone administration on cardiac function and angiotensin II receptor function in rats with isoproterenol-induced heart failure.
Methods. — Wistar rats were divided randomly into control and heart failure groups. The heart failure groups were further divided into the following groups: castration; castration + testosterone replacement; and sham castration. Echocardiography and haemodynamic measurements were used to evaluate cardiac function. Cardiocyte apoptosis and fibrosis were determined using terminal deoxyribonucleotide transferase-mediated dUTP nick-end labelling (TUNEL) staining and Masson's Trichrome staining, respectively. Angiotensin II receptor (AT1 and AT2) messenger ribonucleic acid (mRNA) expression levels were assayed using real-time reverse

Abbreviations: GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HF, heart failure; LV, left ventricle/ventricular; MRNA, messenger ribonucleic acid; POD, peroxidase; PVDF, polyvinylidene difluoride; RAAS, renin-angiotensin-aldosterone system; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; TUNEL, terminal deoxyribonucleotide transferase-mediated dUTP nick-end labelling; VW/BW, ventricular weight/body weight.

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transcriptase-polymerase chain reactions, while Western immunoblotting was used to estimate Bcl-2 protein expression levels.
Results. — Castration significantly increased cardiomyocyte apoptosis and fibrosis that was normally induced by isoproterenol ($P < 0.05$). AT2 receptor mRNA expression in the castration group was increased and Bcl-2 protein expression was decreased compared with the castration + testosterone replacement group ($P < 0.05$).
Conclusion. — These data suggest that androgen therapy could play an important role in pathophysiological changes in heart failure and have beneficial effects for its treatment.

MOTS CLÉS
Testostérone ;
Insuffisance cardiaque ;
Récepteur à l'angiotensine II ;
Apoptose ;
Fibrose

Résumé
Justification. — Le système rénine-angiotensine-aldostérone joue un rôle important dans la physiopathologie et l'apparition de l'insuffisance cardiaque. De nombreuses études ont rapporté les bénéfices de l'administration de testostérone dans l'insuffisance cardiaque. Cependant, les mécanismes des essais induits par la testostérone dans l'insuffisance cardiaque sont mal connus.
Objectif. — Déterminer les effets de la castration et de l'administration de testostérone sur la fonction cardiaque et les récepteurs à l'angiotensine II dans un modèle expérimental de rats avec induction d'une insuffisance cardiaque par l'isoprotérénol.
Méthode. — Les rats Wistar ont été divisés de façon randomisée en groupe témoin et en groupe insuffisance cardiaque. Le groupe insuffisance cardiaque a été divisé secondairement en sous-groupes : castration ; castration + substitution testostérone ; et castration *sham*. L'évaluation échocardiographique et hémodynamique de la fonction cardiaque a été effectuée. L'apoptose et la fibrose ont été déterminées en utilisant la transferase déoxyribonucléotide terminale : (TUNEL) et la coloration trichrome Masson respectivement. L'expression des ARM messagers des récepteurs de l'angiotensine II (AT1 et AT2) a été évaluée en utilisant les techniques de PCR sur la *transcriptase-polymerase reverse*, alors que l'immuomarquage Western a été utilisé pour évaluer les niveaux d'expression de la protéine Bcl-2.
Résultats. — La castration augmente de façon significative l'apoptose des cardiomyocytes ainsi que la fibrose induit par l'isoprotérénol ($p < 0,05$). L'expression des ARN messager du récepteur à l'angiotensine 2 AT2 dans le groupe castration est augmentée alors que l'expression de la protéine Bcl-2 est diminuée, comparativement au groupe castration + administration de testostérone ($p < 0,05$).
Conclusion. — Ces résultats suggèrent que la thérapie androgénique pourrait jouer un rôle important dans les modifications physiopathologiques observées dans l'insuffisance cardiaque et avoir des effets bénéfiques pour son traitement.

Background

Chronic HF is a major health problem throughout the world and a leading cause of morbidity and mortality [1]. Sex differences exist when determining the cause of cardiovascular diseases. Most studies have examined these sex differences by focusing on the effects of oestrogen on cardiovascular function. However, numerous additional studies have indicated that androgens can also have an effect on cardiovascular function. Testosterone levels are decreased in men with HF, while testosterone replacement therapy has been associated with significant increases in cardiac output and improved functional capacity as well as reduced symptoms in men with HF [2,3]. The therapeutic benefits of testosterone in those with chronic HF can be attributed to a number of factors. For example, testosterone has vasodilatory properties and acute administration has been shown to lower peripheral vascular resistance, reduce cardiac afterload and increase the cardiac index. In addition, testosterone can modulate immune responses and improve insulin resistance while also exerting its effects on coagulation, obesity, endothelial function and alterations in skeletal muscle [3—5]. However, the exact mechanisms underlying these effects mediated by testosterone on HF remain unclear.

The RAAS is known to play an important role in the pathophysiology and development of HF. RASS activity is increased in patients with HF, which could lead to cardiac remodelling and sympathetic activation. Both angiotensin II receptor type 1 (AT1 receptor) and angiotensin II receptor type 2 (AT2 receptor) are expressed in the heart, with localization on cardiomyocytes [6]. A number of studies have indicated that angiotensin II receptor expression is altered in the hearts of animal models with HF. Moreover, Nio et al. [7] reported that expression of both AT1 and AT2 receptor mRNA was upregulated in the infarcted and non-infarcted portions of the LV following coronary ligation.

A number of interactions between androgens and RAAS have been shown to occur at several organ sites. Androgens and androgen receptor systems are known to exert protective effects on angiotensin II-induced vascular remodelling [8]. Androgens have also been shown to affect AT1a receptor mRNA abundance in the abdominal but not thoracic aortas

of male mice [9]. Therefore, the purpose of the present study was to determine whether changes in the androgen environment could induce alterations in the expression and activity of heart angiotensin II receptors in HF. Furthermore, we proposed that these activities of angiotensin II receptors are involved in the development of fibrosis and apoptosis in cardiomyocytes in HF.

Methods

All procedures were performed and approved in accordance with the Institutional Animal Care and Use Committee at Harbin Medical University. Male Wistar rats were obtained from the Laboratory Animal Centre of Harbin Medical University (Harbin, China). All animals were maintained on standard rat chow and water ad libitum and were housed in the Laboratory Animal Centre under conditions of controlled ambient temperature (22—24 °C) with a light-dark cycle of 12 h.

Castration and hormone replacement

Seventy sexually mature male rats (7 weeks of age, 160—220 g) were used in this study. Ten rats were randomly selected for the control group. The remaining 60 were divided into three equal groups randomly: castration + placebo (Cas+P); sham castration (S-Cas); and castration + testosterone replacement (Cas+T); Surgeries were performed while the rats were maintained under pentobarbital sodium anaesthesia (60 mg/kg). The rats were placed in a supine position and the testes were removed via a midline incision in the lower abdominal wall. Sham-operated rats received a midline incision that was immediately closed. For rats receiving testosterone propionate, replacement was initiated on the first day following castration. Testosterone propionate (amino acids, P.F., Tianjin, China) was administered once daily (2 mg/kg subcutaneously) for 10 weeks. Placebo (saline) was administered at the same dosage. The dosage and duration of treatments were based upon previously described protocols and data from preliminary experiments in our laboratory [10].

Induction of heart failure

Two weeks postcastration, isoproterenol (Sigma-Aldrich, St. Louis, MO, USA) was administered (340 mg/kg/day, subcutaneously) on two consecutive days. Excluding controls, all rats received an injection of isoproterenol. Eight weeks later, the numbers in the four groups were: Cas+P ($n = 8$); S-Cas ($n = 9$); Cas+T ($n = 10$); control group ($n = 10$).

Echocardiography and haemodynamic measurements

Ten weeks postcastration and hormone replacement, transthoracic echocardiography was performed while the rats were maintained under pentobarbital sodium anaesthesia (60 mg/kg). Two-dimensional and M-mode images were obtained using a 10 Mhz transducer connected to an ultrasonic echocardiographic system (Acuson Sequoia 512; Siemens AG, Erlangen, Germany). Systolic and diastolic dimensions of the LV were obtained from the M-mode view. Ejection fraction was calculated as follows: ejection fraction (%) = [(LVDV−LVSV)/LVDV] × 100, where LVDV and LVSV are left ventricular end-diastolic and end-systolic volumes, respectively. All variables were measured in triplicate and averaged. The investigator who performed the echocardiograms was blinded to the treatment group.

Haemodynamic measurements were also obtained for the estimation of cardiac function. The rats were anaesthetized using pentobarbital sodium (60 mg/kg) and allowed to breathe room air spontaneously. A 2 Fr micromanometer-tipped catheter filled with heparinized saline was fed through the right carotid artery into the LV and connected to a pressure transducer (model SPR-407; Miller Instruments, Houston, TX, USA). During this procedure, LV systolic and diastolic pressures and maximal rates of rise and fall in LV pressure were recorded. Resting haemodynamic measurements were performed in triplicate at 5 min intervals and the average values of these triplicates were used for statistical analyses.

Tissue preparation and histological analysis

Following haemodynamic measurements, harvested cardiectomy was performed and the hearts were weighed. A small portion of the heart was snap frozen in liquid nitrogen and stored at −80°C until biochemical and molecular analyses; a second portion of the heart was fixed in 4% paraformaldehyde overnight. Following fixation, samples were embedded in paraffin and 4 μm sections were cut and stained. In order to calculate the ratio of the interstitial fibrosis area in the LV, samples were stained with Masson's Trichrome and 10 fields were selected randomly from five individual sections. The percentage of fibrotic tissue infiltration in the LV was calculated as follows: (fibrotic tissue area)/(fibrotic tissue area + myocyte area) × 100%.

Analysis of apoptosis

Cardiomyocyte apoptosis was measured using an in situ TUNEL assay. TUNEL staining was performed according to the manufacturer's instructions in the In Situ Cell Death Detection Kit, POD (Roche, Basel, Switzerland). In brief, the tissue sections were deparaffinized and rehydrated using xylene and graded ethanol dilutions with a final rinse in distilled water. The tissue sections were then pretreated with 20 mg/L proteinase K for 30 min and incubated in the TUNEL reaction mixture in a humidified cabinet for 60 min at 37 °C. The tissue was then washed in 0.1 M phosphate buffer saline in triplicate for 5 min and incubated in converter-POD for 30 min at 37 °C. Finally, the tissues were washed in 0.1 M phosphate buffer saline in triplicate for 5 min and stained with a diaminobenzidine-POD substrate (Boster, Wuhan, China). Tissue sections were examined with a microscope at × 400 magnification and at least 100 cells were counted in 10 evenly spaced fields. The percentage of apoptotic cells was identified as the apoptotic index. All measurements were performed in a blind manner.

Real-time reverse transcriptase-polymerase chain reaction

Total RNA was isolated by the TRIZOL technique (Takara, Otsu, Shiga, Japan) according to the manufacturer's instructions. Samples were quantified with a spectrophotometer at 260 nm and integrity was determined using ethidium bromide agarose gel electrophoresis. Reverse transcription was performed using the PrimeScript™ reverse transcription reagent kit (Takara, Otsu, Shiga, Japan). To examine mRNA levels of the AT1 receptor (primer sequences 5′-CATCGTCCACCCAATGAAGTC-3′ and 5′-GGGAACAAGAAGCCCAGAAT-3′) and AT2 receptor (primer sequences 5′-CACAAACCGGCAGATAAGCA-3′ and 5′-CAGGTCCAAAG-AGCCAGTCATA-3′) quantitatively, real-time reverse transcriptase-polymerase chain reaction amplification was performed using a SYBR Premix Ex Taq™ (Takara, Otsu, Shiga, Japan). Expression of GAPDH (primer sequences 5′-CAACG-ACCCCTTCATTGACC-3′ and 5′-GACGCCAGTAGACTCCACGAC-3′) was measured as an internal control for sample variations in the reverse transcription reaction.

Western Blot analyses

Bcl-2 protein expression was measured using Western immunoblotting as previously described [10]. In brief, cardiac tissues were scraped into 0.3 mL lysis buffer (1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulphate [SDS], 100 μg/mL phenylmethylsulphonyl fluoride and 30 μg/mL aprotinin) and incubated for 30 min on ice. Protein concentrations were measured using a Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, CA, USA). Fifty micrograms of protein were mixed and boiled in SDS polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer for 5 min and then separated on SDS-PAGE gels (Bio-Rad, Hercules, CA, USA). Separated proteins were transferred to a PVDF membrane and were probed with polyclonal anti-Bcl-2 (1:1000) and anti-GAPDH antibodies (1:1000) (Santa Cruz Biotechnology, CA, USA) to detect target protein expression. The relative amount of Bcl-2 was determined by densitometry using ImageQuant software, with protein levels of GAPDH used as an internal control.

Statistical analyses

Data are presented as mean ± standard error of the mean. After establishing homogeneity of variance and normal distribution, a one-way analysis of variance was performed to determine differences among groups. If the analysis of variance was determined to be significant, the Student-Newman-Keuls test was used for post hoc pairwise comparisons. Statistical significance was defined as $P < 0.05$. Statistical analyses of data were performed using SPSS statistical software (version 14.0).

Results

Animal mortality

Mortality rates in the Cas+P, S-Cas and Cas+T groups were 60, 55 and 50%, respectively, following isoproterenol administration. There were no significant differences between these three groups ($P > 0.05$).

Body and heart weights

Left ventricular weight normalized to body weight (VW/BW, mg/g) was significantly increased in each of the HF groups (all $P < 0.05$, Table 1) compared with in the control group. Although not statistically significant, castration tended to increase VW/BW, which was alleviated with testosterone replacement.

Effect of testosterone on cardiac function

Rats in each of the treatment groups exhibited a dilated LV, as indicated by an increased LV end-diastolic diameter ($P < 0.05$, Table 1). Ejection fraction was also altered within each treatment group ($P < 0.05$, Table 1). LV end-diastolic diameter was significantly increased in castrated compared with sham-operated rats ($P < 0.05$), which was

Table 1 Effect of testosterone on cardiac function.

	Control ($n = 10$)	Cas+T ($n = 10$)	Cas+P ($n = 8$)	S-Cas+P ($n = 9$)
VW/BW (mg/g)	2.55 ± 0.14	2.76 ± 0.09*	2.85 ± 0.17*	2.80 ± 0.15*
LVEDD (mm)	5.3 ± 0.2	5.7 ± 0.4*,**	6.2 ± 0.4*	5.8 ± 0.3*,**
EF (%)	84.3 ± 3.8	71.3 ± 5.5*,**	61.0 ± 5.6*	62.6 ± 5.4*
HR (beats/min)	304 ± 33	352 ± 31*,**	385 ± 28*	374 ± 41*
LVESP (mmHg)	109.1 ± 4.1	96.9 ± 5.7*,**	85 ± 6.4*	90.3 ± 4.1*
LVEDP (mmHg)	4.9 ± 1.3	14.9 ± 2.8*,**	22.8 ± 5.4*	15.5 ± 2.2*,**
$+dP/dt_{max}$ (mmHg/s)	3966.6 ± 156.4	3327 ± 204*,**	2786 ± 153.9*	3128 ± 234.6*,**
$-dP/dt_{max}$ (mmHg/s)	−3730 ± 170.8	−3115.2 ± 216*,**	−2648.9 ± 169.3*	−2978.2 ± 208.6*,**

All data are mean ± standard error of the mean. Cas+P: castration + placebo; Cas+T: castration + testosterone replacement; + dP/dt: maximum peak rate of rise in left ventricular pressure; $-dP/dt$: maximum peak rate of fall in left ventricular pressure; EF: ejection fraction; HR: heart rate; LVEDD: left ventricular end-diastolic diameter; LVEDP: left ventricular end-diastolic pressure; LVESP: left ventricular end-systolic pressure; S-Cas+P: sham castration + placebo; VW/BW: ventricular weight/body weight.

* $P < 0.05$ versus control group.

** $P < 0.05$ versus Cas+P group.

reversed with testosterone replacement ($P < 0.05$). Testosterone replacement also improved cardiac function in rats with HF ($P < 0.05$).

Rats with induced HF exhibited increased LV end-diastolic pressure and heart rate, and decreased LV end-systolic pressure and peak rate of change in LV pressure (dP/dt), compared with controls (all $P < 0.05$, Table 1). The LV end-diastolic pressure of rats receiving testosterone replacement was significantly decreased compared with placebo-treated animals ($P < 0.05$).

Effects of testosterone on apoptosis and cardiac fibrosis

Fig. 1 shows TUNEL staining following treatment in all groups ($n = 8$). TUNEL-positive nuclei were observed in the HF versus control groups ($P < 0.05$). The number of TUNEL-positive nuclei in cardiomyocytes was significantly lower in the Cas+T and S-Cas groups than in the Cas+P group ($P < 0.05$). Furthermore, cardiomyocytes from the Cas+T group were more predominant than in the S-Cas group ($P < 0.05$). Testosterone replacement abrogated the development of fibrosis compared with animals in the Cas+P and S-Cas groups ($P < 0.05$, Fig. 2).

Effects of testosterone on AT1 receptor and AT2 receptor gene expression

Although there were no significant differences in AT2 receptor mRNA levels between the Cas+T, S-Cas and control groups, HF tended to increase AT2 receptor mRNA expression (Cas+T/control = 1.41, S-Cas/control = 2.2; Fig. 3B).

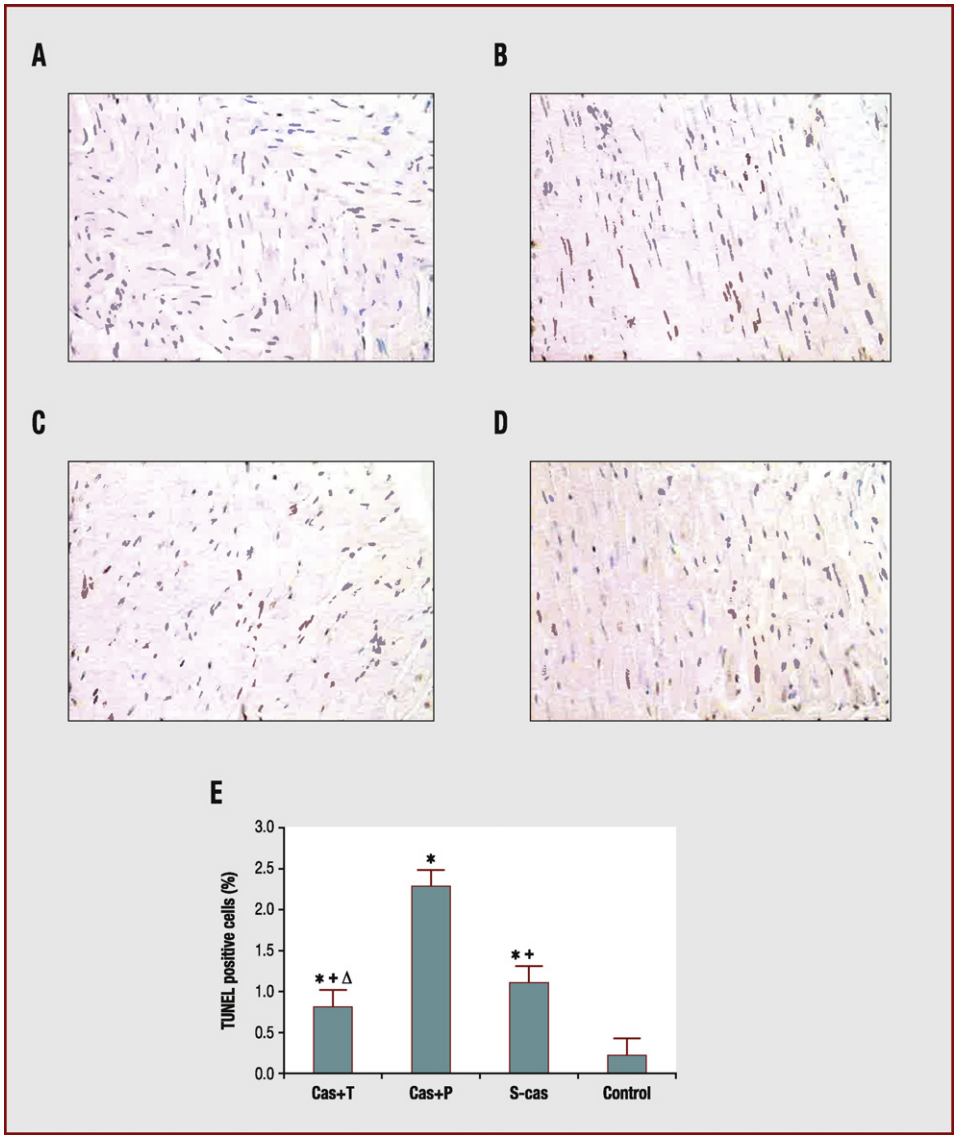

Figure 1. Effect of heart failure and testosterone therapy on cardiac apoptosis. (A—D) representative photographs for myocardial TUNEL staining ($n = 8$): (A) control; (B) Cas+P group; (C) S-Cas group; (D) Cas+T group. (E) quantitative analysis of TUNEL-positive cells in the four groups: control group 0.22 ± 0.07; Cas+P group 2.28 ± 0.48; S-Cas group 1.1 ± 0.22; Cas+T group 0.82 ± 0.12. Data are expressed as mean $\pm$ standard error of the mean. $^{*}P < 0.05$ versus control group; $+P < 0.05$ versus Cas+P group; $\Delta P < 0.05$ versus S-Cas group. Cas+P: castration + placebo; Cas+T: castration + testosterone replacement; S-Cas: sham castration.

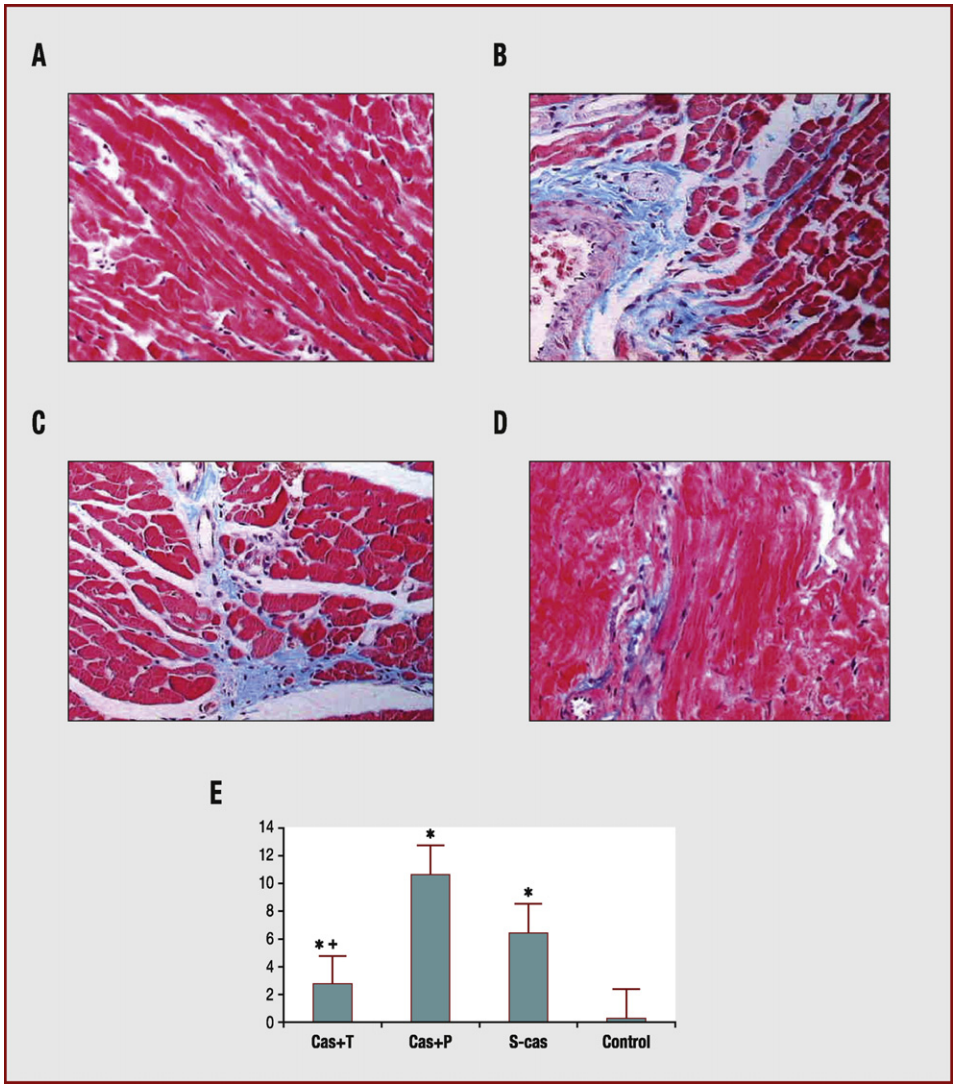

Figure 2. Representative Masson's Trichrome-stained images ($n = 8$): fibrotic tissue, blue; residual myocytes, red. (A) control group; (B) Cas+P group; (C) S-Cas group; (D) Cas+T group. (E) Percentage of fibrotic tissue infiltration in the left ventricle from all groups: control group 0.35 ± 0.17; Cas+P group 10.7 ± 2.54; S-Cas group 6.5 ± 1.98; Cas+T group 2.55 ± 0.52. Data are expressed as mean ± standard error of the mean. $^{*}P < 0.05$ versus control group; $+P < 0.05$ versus Cas+P and S-Cas groups. Cas+P: castration + placebo; Cas+T: castration + testosterone replacement; S-Cas: sham castration.

Furthermore, the Cas+P group had increased AT2 receptor mRNA levels compared with the control group ($P < 0.05$, Fig. 3B), while the Cas+T group had reduced AT2 receptor mRNA levels compared with the Cas+P group ($P < 0.05$). HF increased AT1 receptor mRNA expression ($P < 0.05$) but there were no statistically significant differences in AT1 receptor mRNA expression between the Cas+T, Cas+P and S-Cas groups. ($P > 0.05$, Fig. 3A).

Effects of testosterone on Bcl-2 protein expression

Western immunoblot analysis of Bcl-2 protein expression is shown in Fig. 3C. Isoproterenol administration caused a decrease in Bcl-2 protein expression ($P < 0.05$). In the Cas+P group, Bcl-2 protein expression was decreased further than in the S-Cas group ($P < 0.05$), while testosterone replacement increased Bcl-2 protein expression ($P < 0.05$).

Discussion

In the present study, we found that:

- castration worsened cardiac function of rats with HF and testosterone replacement could reduce this decrease in cardiac function;
- testosterone replacement reduced the extent of myocardial fibrosis in HF induced with isoproterenol;
- castration resulted in an elevation in AT2 receptor expression but these changes were reduced with testosterone replacement;
- testosterone replacement decreased cardiac cell apoptosis that was induced with isoproterenol and increased Bcl-2 protein expression.

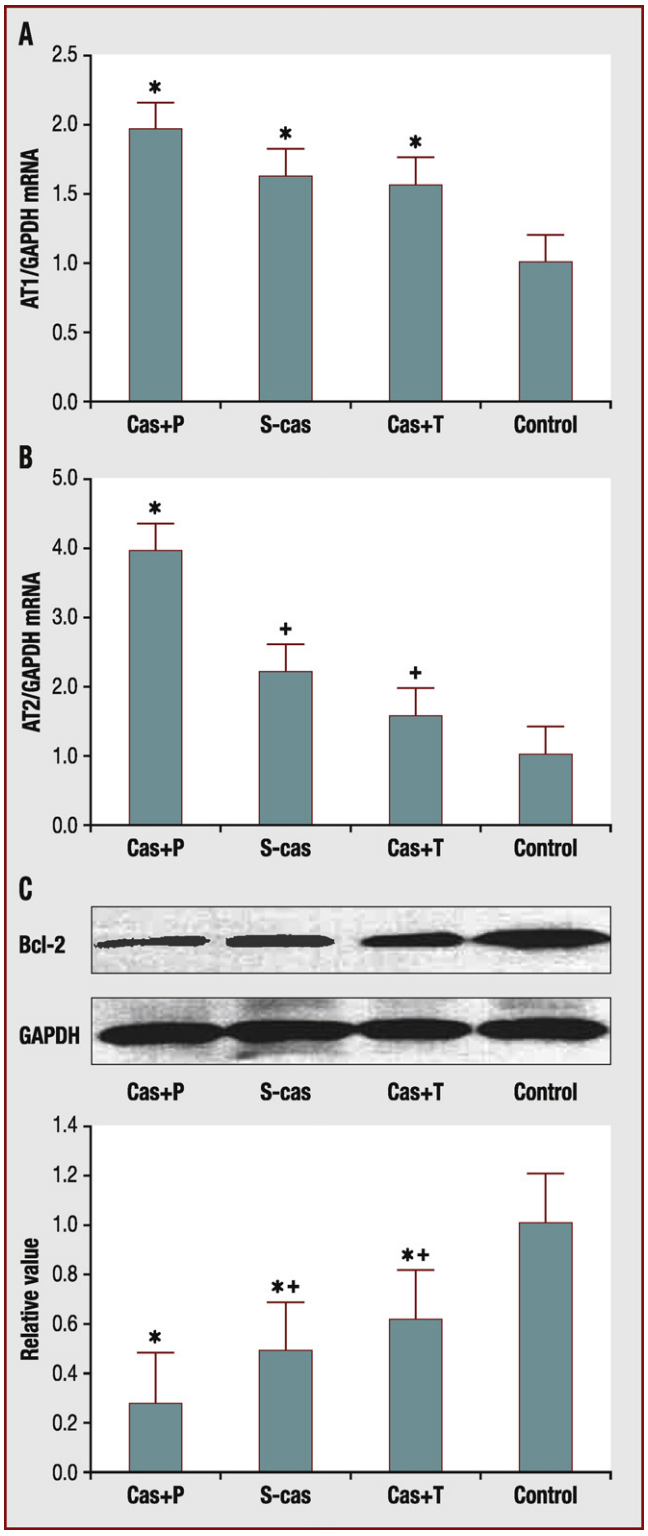

Figure 3. Effect of testosterone on levels of expression of angiotensin II receptor (AT1 and AT2) mRNA and Bcl-2 protein. (A) levels of expression of AT1 receptor mRNA in all groups; (B) levels of expression of AT2 receptor mRNA in all groups; (C) levels of expression of Bcl-2 protein in all groups. Control group ($n = 10$); Cas+P group ($n = 8$); S-Cas group ($n = 9$); Cas+T group ($n = 10$). *$P < 0.05$ versus control group; +$P < 0.05$ versus Cas+P group. Cas+P: castration + placebo; Cas+T: castration + testosterone replacement; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; S-Cas: sham castration.

Isoproterenol is a non-selective β_1- and β_2-adrenoceptor agonist that can exert toxic effects through multiple mechanisms, including an increase in intracellular cyclic adenosine monophosphate, calcium overload, alterations in electrophysiological properties of cardiomyocytes, ischaemia and increased oxidative stress [11,12]. Isoproterenol at high doses results in an extensive amount of cardiomyocyte necrosis [13]. Similar to results from experimental myocardial infarction, significant cardiomyocyte loss mediated by isoproterenol administration is obtained predominantly in the LV and represents the initial insult that triggers the onset of HF. Weber et al. [14] reported that catecholamines administered at high doses resulted in myocardial fibrosis and significant alterations in thick and thin collagen fibres. Accordingly, this simple model can be useful for examining therapeutic potential in chronic HF.

The protective effects of oestrogen on the cardiovascular system have been extensively studied; however, few clinical studies have examined the relationships between androgens and cardiovascular disease. A negative correlation between testosterone levels and cardiac function has previously been reported [15]. Men with HF have lower-than-normal testosterone levels and reduced levels of serum anabolic hormones, constituting strong markers for poor prognosis, independent of conventional risk predictors. Furthermore, testosterone deficiency is known to be positively correlated with cardiac output in patients with chronic HF; a significant improvement in this variable has been observed following testosterone replacement therapy [2,3]. Golden et al. reported that androgens are capable of directly enhancing cardiomyocyte function in the absence of other cardioregulatory haemodynamic and humoral factors [16]. Our results are consistent with these reports, in that testosterone deprivation decreased further cardiac dysfunction, while testosterone replacement therapy improved cardiac function in isoproterenol-induced HF. In vivo studies have shown that augmented cardiac contractile velocity in orchidectomized animals that were given testosterone was associated with a significant alteration from the slower myosin heavy chain β to the faster myosin heavy chain α and enhanced L-type calcium-channel mRNA accumulation [17].

The RAAS plays an important role in the progression of HF. Angiotensin II binds to angiotensin II receptors in the heart and exerts other non-haemodynamic effects [18,19]. Rogers et al. [20] reported that both castration and dihydrotestosterone (25 mg) did not alter glomerular AT1 receptor binding in rats, while a high dose of dihydrotestosterone (200 mg) profoundly decreased AT1 receptor binding. Similarly, Song et al. [21] found that neither castration nor castration + testosterone treatment affected AT1 receptor mRNA or protein expression. In the present study, we observed that neither castration nor testosterone replacement affected AT1 receptor mRNA expression, consistent with the studies noted above.

Although both AT1 and AT2 receptors have seven transmembrane domains typical of G protein-coupled receptors, they have different functional properties and signal transduction mechanisms. Ichihara et al. [22] demonstrated that the AT2 receptor is related to cardiac fibrosis and collagen deposition induced by angiotensin II infusion. Brilla et al. [23] reported that angiotensin II stimulated collagen synthesis through both the AT1 and AT2 receptors in rat cardiac

fibroblasts and that angiotensin II-induced inhibition of collagenase activity was mediated by AT2 receptors. In the present study, we determined that castration-induced elevated AT2 receptor mRNA expression in rats with HF, while testosterone replacement suppressed this effect. Likewise, cardiac fibrosis was decreased in the testosterone replacement group compared with in the castration alone group. Nakazawa et al. [24] also reported that castration increased AT2 receptor mRNA levels while testosterone administration reversed this effect in the urinary bladder. Moreover, Natoli et al. [25] found that collagen deposition was reduced in human aortic smooth muscle cells incubated with testosterone. Changes in AT2 receptor mRNA expression in HF accompanied by changes in the androgen environment are considered to be among the important phenomena in cardiac function. Androgen action on the epididymal angiotensin system has been suggested to be at least partially mediated through cyclooxygenase-1 expression [26]. Further examinations are necessary to clarify the crosstalk mechanisms between the angiotensin and androgen systems in HF.

Apoptosis plays a crucial role in normal development as well as in pathophysiology of various tissues. AT2 receptor stimulation was demonstrated to induce apoptosis in many cell types. In vivo studies have demonstrated that AT2 receptors mediate vascular mass regression through stimulation of smooth muscle cell apoptosis [27,28]. Mechanisms responsible for AT2 receptor-mediated apoptosis appear to be complex, particularly because AT2 receptors activate tyrosine phosphatases, including mitogen-activated protein kinase phosphatase-1 (MKP-1), and inactivate the signal-related extracellular mitogen-activated protein kinases ERK1 and ERK2, resulting in Bcl-2 dephosphorylation and upregulation of Bax [29]. Boissonneault [30] reported that gonadectomy-induced apoptosis of the rat levator ani muscle, while Fan and Robaire [31] demonstrated that orchidectomy induced apoptosis in the epididymis, with androgen therapy partially reversing this change. In the present study, isoproterenol-induced cardiac cell apoptosis and castration further aggravated this process. Testosterone treatment decreased cell death induced through castration. These findings suggest that alterations in the androgen environment have an effect on cardiac cell apoptosis. As these changes in apoptosis and Bcl-2 are consistent with AT2 receptor changes, we conclude that testosterone affected apoptosis through the AT2 receptor.

Conclusion

In conclusion, castration increased AT2 receptor expression, cardiac fibrosis and cardiac cell apoptosis in rats with HF, which were reduced following testosterone replacement. These changes were accompanied by significant improvements in LV function. Our findings imply that androgen replacement may have beneficial effects for male HF with androgen deficiency by altering the RASS system.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgments

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